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Pathology of the skeleton of Indo-Pacific bottlenose dolphins *Tursiops aduncus*: a comparison of adjacent gulfs in South Australia

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ABSTRACT: Studies of skeletal pathology of marine mammals can contribute to conservation measures, yet few have focused on causative factors. Museums hold vast collections of skeletons relevant to this knowledge gap. Indo-Pacific bottlenose dolphin *Tursiops aduncus* carcasses (n = 162) were collected from Gulf St Vincent (GSV) and Spencer Gulf (SG), South Australia (SA), between 1988 and 2013, and post-mortem examinations were carried out. After preparing skeletons, their gross pathology was classified into 4 categories: lytic lesions, degenerative lesions, fractures and malformations. Comparisons were made between gulfs based on pathology type, relative age, period of collection and cause of death (anthropogenic vs. non-anthropogenic). Pathology prevalence (76%) was higher than reported in other studies but observed pathologies were similar. More than one pathology was observed in 88% of dolphins that had pathology. Vertebrae were often the site of pathology. Prevalence of lesions were: fractures (82%), lytic lesions (75%) including spondylo-osteomyelitis and osteomyelitis, degenerative lesions (57%) including spondylosis deformans, and malformations (17%). Prevalence of pathology increased with dolphin relative age, and GSV dolphins had more pathology than those in SG. In SG, anthropogenic cases had more pathology than non-anthropogenic cases, and dolphins collected after 2000 had more degenerative lesions than those collected before 2000. There were more malformations in SG dolphins than those from GSV, although this was not statistically significant. In one anomalous case, an individual was found with 5 detached transverse processes on 4 lumbar vertebrae. Heavy metal toxicity reported for *T. aduncus* from SG may be related to the malformations reported in this study.

KEY WORDS: Skeletal pathology · Lytic lesions · Degenerative lesions · Fractures · Malformation · Indo-Pacific bottlenose dolphin

INTRODUCTION

Pathology of the skeleton has been well studied in domestic and laboratory mammals (Hansen 1952, Thompson 2007) and wild terrestrial mammals (Bulstrode et al. 1986, Rothschild & Woods 1991, Rothschild et al. 1998, Hufschmid et al. 2015). The number of studies of cetacean skeletal pathology have been increasing (Kompanje 1999, Galatius et al. 2009, Fettuccia et al. 2013, Bertulli et al. 2015, Weir & Wang 2016). The causes of skeletal pathology include infectious disease, trauma, aging, metabolic disorders, degenerative diseases, toxic contaminants and developmental disorders, which can be classified as acquired, congenital or anthropogenic in origin (Kompanje 1999, Van Bressem et al. 2007, San

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Martín et al. 2016). In serious cases, some forms of skeletal pathology may result in death, while in others it may be debilitating (Thompson 2007).

Diseases of bone can be studied either by gross (skeletal) pathology or histopathology (bone pathology), the latter requiring preserved, fresh material. Kompanje (1995a, 1999) described cetacean skeletal pathology as it relates to diagnosis in 47 species of Odontoceti and 10 Mysticeti. Van Bressem et al. (2007) expanded on these studies by investigating aetiology in 12 odontocetes, including lytic lesions, degenerative lesions, fractures and malformations. These studies provide a good basis for the present study.

Studies of cetacean skeletal pathology have focused on diagnosis and description of pathologies for a limited number of small cetaceans. Delphinids have been better studied than other groups. Some studies investigated only skull pathology (Pascual et al. 2000, Van Bressem et al. 2006, Loch et al. 2011), while others included skulls and postcranial elements (De Smet 1977, Kompanje 1995a, Van Bressem et al. 2007, Fettuccia et al. 2013) or only postcranial specimens (Walker et al. 1986, Kompanje 1995b, Berghan & Visser 2000, Galatius et al. 2009, Laeta et al. 2010).

Few studies of gross skeletal pathology have been undertaken on Australian cetaceans. Pathology of a minke whale *Balaenoptera acutorostrata* and a humpback whale *Megaptera novaeangliae* neonate from Queensland were described, with the conclusion that the aetiology for both was trauma during parturition (Paterson & Van Dyck 1996). A single Bryde's whale *Balaenoptera edeni* from Queensland was documented with spondylitis deformans (Paterson 1984). Abnormalities of the skull and vertebral fractures were reported in a common dolphin *Delphinus delphis* from Victoria (Dixon 1984). Lavery et al. (2009) found preliminary evidence for heavy metal effects on the microstructure of bone in Indo-Pacific bottlenose dolphins *Tursiops aduncus* in South Australia (SA).

Spencer Gulf (SG) and Gulf St Vincent (GSV) in SA are large, semi-enclosed, inverse estuaries (Nunes Vaz et al. 1990) that are ideal 'living laboratories' for studying biological processes (Bye & Kämpf 2008). The 2 gulfs are similar oceanographically but differ in terms of human activities. There are more heavy industrial developments producing toxic contaminants in SG but less urban development (Harbison 2014). GSV has fewer currents than SG (Bye & Kämpf 2008). Because there is minimal fresh water input and water depth is generally less than 30 m, these marine embayments build up sediments and pollutants which bioaccumulate and can be harmful to long-lived organisms such as marine mammals. Input of heavy metals from industrial and urban activities is exacerbated by hypersaline conditions in the northern reaches of the gulfs. *T. aduncus* is a resident and philopatric species, with restricted genetic exchange between the populations in each gulf (Bilgmann et al. 2007). Being a benthic feeder (Gibbs et al. 2011), it is at risk of heavy metal toxicity (Lavery et al. 2009). Stranding records (Segawa & Kemper 2015) suggest that this species is common in both gulfs.

The present study examined a large sample of complete skeletons of *T. aduncus* from SA to document types, aetiology and prevalence of skeletal pathology. It is the first study of small cetaceans to employ statistical methods to investigate patterns in skeletal pathology related to relative age, anthropogenic circumstances and period of collection. Comparisons were also made between the 2 gulfs.

MATERIALS AND METHODS

Indo-Pacific bottlenose dolphins *Tursiops aduncus* carcasses (n = 162) were salvaged from 2 adjacent gulfs, 83 from the GSV bioregion (40 males, 42 females, 1 unknown) and 79 from SG (50 males, 29 females) between 1988 and 2013. The dolphins either stranded live and died, were beach-cast or floating carcasses, or died as bycatch in fisheries and aquaculture gear.

Post-mortem examinations were conducted on 161 carcasses. Data collected included developmental features indicating relative age of young animals (Kemper & Gibbs 2001), sex, body weight, total body length, reproductive organ maturity (Kemper et al. 2014, 2018) and gross pathology.

Carcasses were flensed and the skeletons macerated in warm water (30 to 35°C) for 3 to 5 mo, after which they were scrubbed in soapy water to remove any remaining tissue and the adipocere. Skeletons prepared before 2000 were degreased in boiling trichloroethylene for about 1 d; skeletons prepared after this date were not degreased. Skeletons were registered into the mammal collection of the South Australian Museum in Adelaide (SAMA).

A circumstance of death was assigned to each dolphin using observations at the time of collection and data collected post-mortem. Categories were unknown, live stranded, disease, other natural causes, captured, known entanglement, probable entanglement, other unintentional and intentional killing (see Kemper et al. 2005 for detailed defini-

tions). Anthropogenic circumstances were categorised as entanglements and other unintentional and intentional cases. The remainder were non-anthropogenic.

Specimens were assigned to relative age classes (neonate, calf, juvenile, subadult, and adult) based on physical maturity (the status of epiphyseal fusion of vertebrae), sexual maturity (size and condition of reproductive organs), total body length and developmental features (Kemper & Gibbs 2001). Neonates, calves and juveniles were classified as immature; subadults and adults as mature. Absolute age of one dolphin (M22410) was determined by tooth incremental layers (Kemper et al. 2018).

One of the variables analysed related to when the dolphins were collected; the data being divided into 2 periods of collection: 1988 to 2000 and 2001 to 2013. This was based primarily on there being similar sample sizes for these periods and because post-mortems were more comprehensive after 2001.

Classification of skeletal pathology

Skulls and skeletons were examined to determine the type of pathology and its location on the skeleton. Representative photographs were taken. Pathologies were classified into 4 types following Kompanje (1995a, 1999) and Van Bressem et al. (2007):

(1) Lytic lesions, including osteomyelitis, spondylitis, osteolysis, periodontal lysis and crassicaudiasis. Osteomyelitis, spondylitis, osteolysis, reactive spondyloarthropathy (ankylosing spondylitis) spondylodiscitis and soft tissue ossification were observed in vertebrae, ribs, scapulae and bones of the flipper. These lesions were associated with one or more pores on the surface with the overall rugose appearance. In severe cases, the vertebrae were ankylosed. Periodontal lysis included alveolar osteitis.

(2) Degenerative lesions, including hyperostosis, osteophytes and spondylosis, were generally smooth in appearance. Hyperostosis and osteophytes were found in older dolphins at the periphery of vertebral bodies. Spondylosis includes ossification, which may result in fusion of vertebrae (ankylosing spondylosis) or fusion of the atlas with the occipital condyles, as well as fusion of other parts of the skeleton. Intraspongious disc herniation (Schmorl's nodes) is included in this classification.

(3) Fractures, including both healed and unhealed lesions. Normally the healed fractures formed a callus that protruded from the surface of the bone. Some healed fractures were aligned.

(4) Malformations, which are the result of congenital disruption of skeleton morphology. These abnormal bone formations were identified by missing and/or unfused elements or fusion of adjacent bones, and usually had a smooth surface.

In addition, one anomalous dolphin could not be clearly classified into the above classifications.

Statistical analyses

All statistical tests were performed in Statcel (1998 1st edn; OMS). When comparing frequencies of skeletal pathology with certain variables, a Fisher's exact probability test was used if sample size in any cell was ≤5. This occurred when (1) comparing pathology type (except lytic lesions) and pathology prevalence with both relative age and circumstance of death using data from both gulfs combined, as well as (2) within gulfs, comparing pathology prevalence with relative age and circumstance of death, and type of pathology with circumstance of death. In all other cases, a chi-squared test of independence was used for (1) comparison of pathology type and pathology prevalence between gulfs, (2) combined-gulfs comparison of lytic lesions with relative age, (3) pathology type and pathology prevalence with time period and (4) within-gulfs comparison of prevalence of pathology and type of pathology with time period. Chi-squared goodness-of-fit was calculated with a desktop calculator to test prevalence of skeletal pathology between males and females (Bailey 1959).

RESULTS

A total of 162 Indo-Pacific bottlenose dolphins *Tursiops aduncus* were collected and examined for skeletal pathology; all but one were also examined for gross pathology. The number of dolphins examined for each variable and from each gulf are found in Table 1, and their locations relative to the time period when they were collected (period of collection) in Fig. 1. Both the place where the carcasses were collected and the time of collection were evenly distributed. Since the ratio of males to females was not significantly different ($p = 0.78$, $n = 161$), sexes were combined in subsequent analyses. Data in Table 1 form the basis of comparisons and statistical analyses that follow. Skeletal pathology was observed in 76% (124/162) of *T. aduncus* from SA (Table 1). Of the dolphins that had pathology, 88%

Table 1. Number of *Tursiops aduncus* assigned to variables analysed in the study (excluding 1 dolphin of unknown sex). Circumstance of death numbers exclude dolphins with unknown cause of death: 27 in GSV and 40 in SG. GSV: Gulf St Vincent; SG: Spencer Gulf

	No. examined	No. with pathology	Sex		Age		Period of collection		Circumstance of death	
			M	F	Immature	Mature	1988–2000	2001–2013	Anthropogenic	Non-anthropogenic
GSV	83	71	40	42	45	38	31	52	18	38
SG	79	53	50	29	39	40	39	40	27	12
Total	162	124	90	71	84	78	70	92	45	50

(109/124) occurred in the vertebrae, 55% (68/124) in the skull and 53% (66/124) in the ribs (Table 2).

Categories of skeletal pathology observed

Four types of pathology were recorded (Table 3). Fractures were found in 82% (102/124) of dolphins with pathology, most (91%, 93/102) occurring in the vertebrae. The dorsal portion of the neural spines and the transverse processes were the most common elements fractured, particularly at the caudal end of thoracic and cranial end of the lumbar vertebrae region (Fig. 2). Ribs were the next most commonly fractured bones (19%, 19/102). In total, 14 skulls had fractures, mostly on the rostrum and mandible.

Lytic lesions were the second most prevalent, recorded in 75% (93/124) of dolphins with pathology. Osteomyelitis and/or spondylitis of varying degrees of severity were found in 73 dolphins. Spondylo-osteomyelitis involving ankylosis (Fig. 3) was found in the vertebrae of 7 dolphins, with no particular region favoured. Osteolysis was observed in one skull, expressed as a channel-like fistula in the exoccipital bone (Fig. 4). Periodontal lysis was found in 43 dolphins, both in the mandible and maxilla, mostly in the middle and anterior alveoli. Lytic lesions caused by parasites (e.g. *Crassicauda* sp.) were not observed in the examined skulls.

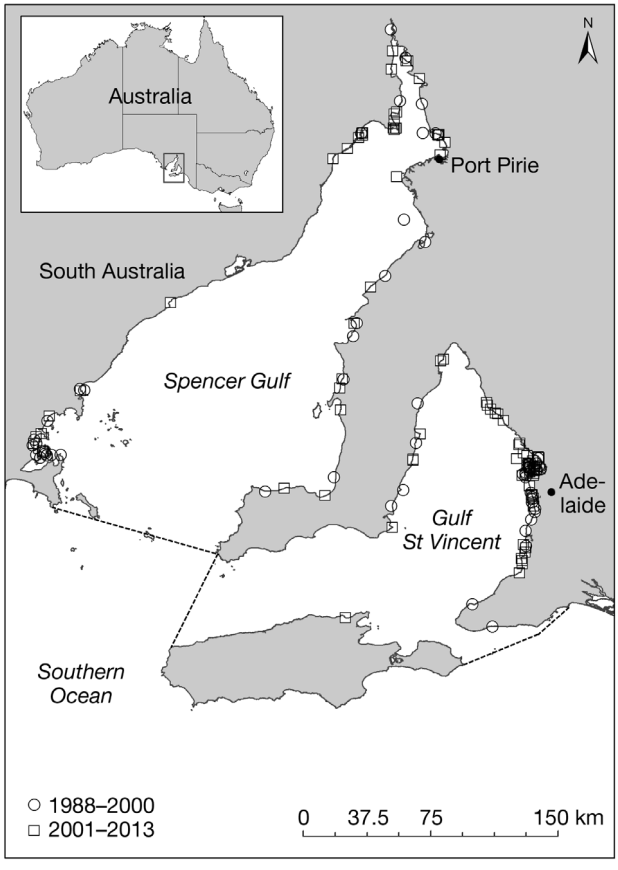

Fig. 1. Coast of South Australia showing locations of *Tursiops aduncus* collected between 1988 and 2013. Dotted lines: limits of gulfs

Table 2. Number of *Tursiops aduncus* with skeletal pathology relative to body region. Some dolphins showed pathology in multiple regions. Rib numbers include vertebral and sternal ribs

	Vertebrae	Skull	Ribs	Flipper	Scapula	Other
GSV	58	41	37	23	17	13
SG	51	27	29	6	7	19
Total	109	68	66	29	24	32

Table 3. Skeletal pathologies recorded in *Tursiops aduncus* from South Australia. Some dolphins showed multiple pathologies

	Lytic lesions	Degenerative lesions	Fractures	Malformations
GSV	55	34	58	8
SG	38	37	44	13
Total	93	71	102	21

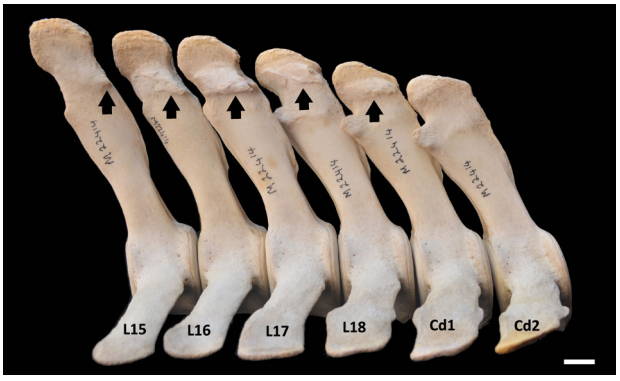

Fig. 2. Healed fractures (arrows) on the neural spines of lumbar and caudal vertebrae of an adult female *Tursiops aduncus* (M22414). Clear discontinuities in the neural spines were seen in posterior view. Left lateral view. Scale bar = 1 cm

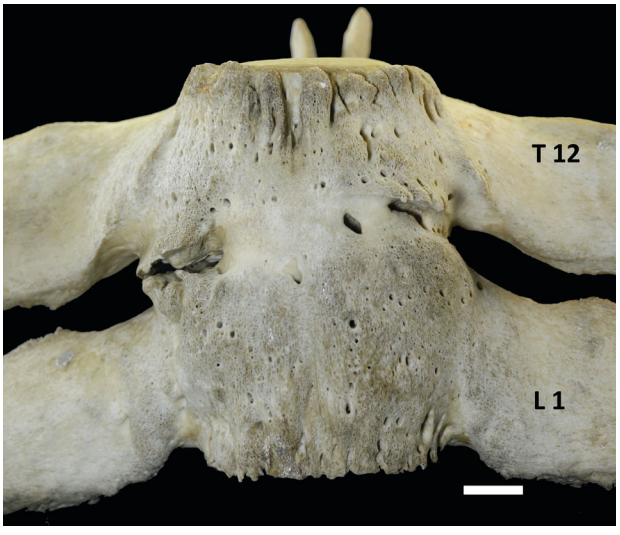

Fig. 3. Lytic lesion (chronic spondylo-osteomyelitis, ankylosis) on vertebrae (T12 and L1) of an adult male *Tursiops aduncus* (M21237). This dolphin also had healed fracture on the neural spines of lumbar vertebrae (L3–5, 8–10) as well as osteoarthritis in the facets between transverse processes of vertebrae T6 and 7 and corresponding ribs. Ventral view. Scale bar = 1 cm

Degenerative lesions were observed in 57% (71/124) of dolphins with pathology (Table 3) and were found mainly in vertebrae (62%, 44/71). Five dolphins had degenerative lesions in multiple regions of the vertebral column, particularly the cervical vertebrae. Hyperostosic lesions were frequently observed on the vertebral body (Fig. 5), often as marginal osteophytes. In addition, 10 dolphins also showed hyperostotic lesions in the temporomandibular joint. Spondylosis deformans of varying degrees of severity was found in the vertebrae of 4 dolphins, of which 2 displayed ankylosis of lumbar or caudal vertebrae.

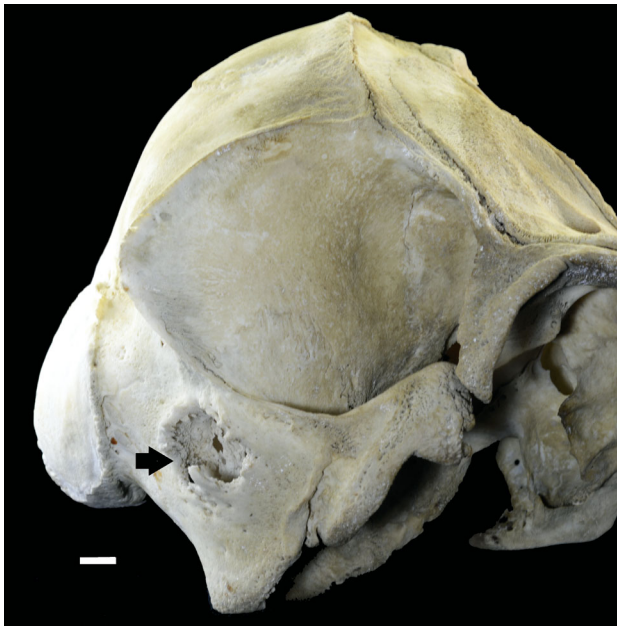

Fig. 4. Lytic lesion on the skull of an adult female *Tursiops aduncus* (M22438). The arrow indicates osteolysis on the exoccipital bone. Right lateral view. Scale bar = 1 cm

Malformations were noted in 17% (21/124) of dolphins with pathology (Table 3). In 5 dolphins, malformation was apparent as fusion of sternal ribs. Four cases of fused sternal ribs were observed unilaterally on the 3rd and 4th or 4th and 5th ribs. The fifth case was bilateral (Fig. 6) and also appeared to lack a third sternebra, which may have been developmentally subsumed into the fused sternal ribs. Six dolphins had unfused vertebral arches in their cervical vertebrae.

In one anomalous case, a 14 yr old female from the northern part of SG (M22410) was not assigned to any of the 4 pathology types discussed above. Four of its lumbar vertebrae (L11–14) had transverse processes (3 on left, 2 on right) that were detached from

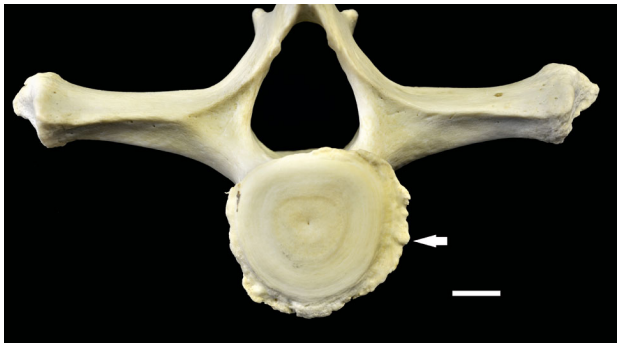

Fig. 5. Degenerative lesion on the thoracic vertebra (T8) of an adult male *Tursiops aduncus* (M20877). Arrow indicates hyperostosis on the vertebral body. Caudal view. Scale bar = 1 cm

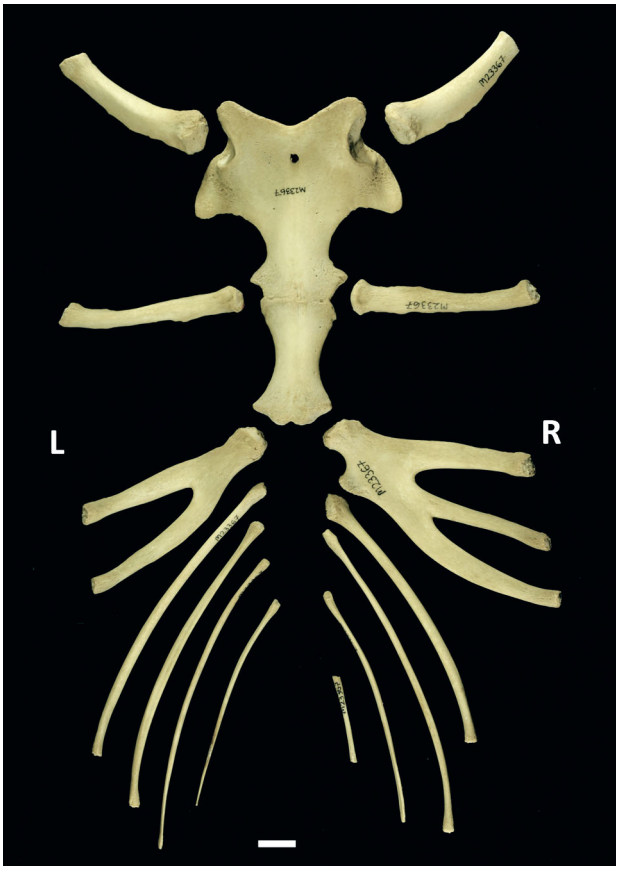

Fig. 6. Malformations of sternal ribs of an adult female *Tursiops aduncus* (M23367). The proximal ends of sternal ribs L3 and 4 and R3–5 were fused. The third sternebrae was not found after maceration. For the illustration, sternal ribs were placed in presumed positions. Dorsal view. Scale bar = 2 cm

Fig. 7. Lumbar vertebrae of a subadult female *Tursiops aduncus* (M22410) with anomalous pathology. Detached transverse processes are associated with their presumed respective vertebral bodies. Neural spines of L12–14 had healed fractures. Dorsal view. Scale bar = 1 cm

the vertebral body. The presumed articular surfaces were smooth-edged and smaller than the same area in adjacent vertebrae (Fig. 7). Healed fractures, with callus formations, were observed near the dorsal end of the neural spines of L12–L14.

Patterns in pathology prevalence

When data on dolphins from both gulfs were combined, trends were observed in some of the variables identified in the study. The prevalence of pathology increased with relative age (Fig. 8); there was a significant difference in prevalence between immature and mature dolphins (Table 4), with individuals from GSV having significantly more skeletal pathology than SG (Fig. 8). There was no difference in prevalence between anthropogenic and non-anthropogenic cause of death nor was there any difference for the period of collection.

When only dolphins with skeletal pathology were analysed, prevalence was also significantly greater in mature compared with immature dolphins for lytic lesions, degenerative lesions and fractures (Table 4), but not for malformation. Also, degenerative lesions had significantly greater prevalence in SG compared with GSV, but not for period of collection or circumstance of death.

When data on dolphins from GSV and SG were analysed separately, age showed a significant relationship in prevalence for all dolphins and for the subsample of only dolphins with pathology. Within each gulf, mature dolphins with skeletal pathology had more lytic lesions, degenerative lesions and fractures than immature ones. For SG, the prevalence of skeletal pathology for all dolphins, including those without pathology, was greater for anthropogenic

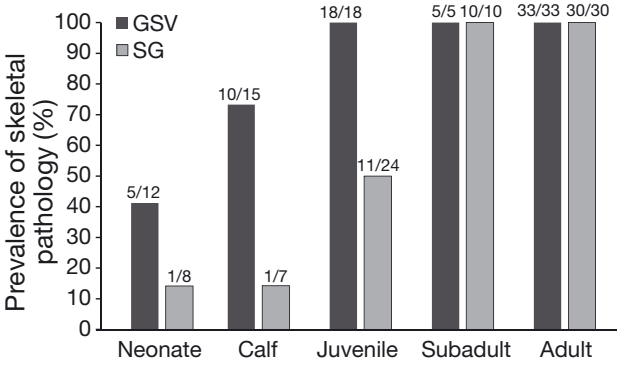

Fig. 8. Prevalence of *Tursiops aduncus* from Gulf St Vincent (GSV) and Spencer Gulf (SG) with skeletal pathology in 5 relative age categories. Numbers above bars: dolphins with pathology/dolphins examined

Table 4. Statistical analyses comparing prevalence of skeletal pathology with variables identified in the study of *Tursiops aduncus* from South Australia. GSV: Gulf St Vincent; SG: Spencer Gulf. Significant levels: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS: not significant. NA: not available. Periods of collection were 1988 to 2000 and 2001 to 2013. Skeletal pathology includes all dolphins; lytic lesions, degenerative lesions and fractures include only dolphins with pathology

	GSV and SG	GSV	SG
Skeletal pathology			
Locality	GSV > SG*	NA	NA
Age	Mature > immature***	Mature > immature***	Mature > immature***
Circumstance of death	NS	NS	Anthropogenic > non-anthropogenic*
Lytic lesions			
Age	Mature > immature***	Mature > immature**	Mature > immature**
Degenerative lesions			
Locality	SG > GSV*	NA	NA
Age	Mature > immature***	Mature > immature***	Mature > immature***
Period of collection	NS	NS	2001–2013 > 1988–2000*
Fractures			
Age	Mature > immature***	Mature > immature***	Mature > immature*

circumstance of death when compared with non-anthropogenic, but this did not apply to GSV. For the data set that included only SG dolphins with pathology, degenerative lesions were more prevalent during 2001 to 2013 than 1988 to 2000 (Table 4). All other comparisons showed no significant differences between variables within each gulf.

DISCUSSION

Marine mammal necropsies have been carried out on thousands of animals worldwide and their skeletons collected, but few have included the follow-up investigation of skeletal pathology (Duignan et al. 2003, Martinez & Stockin 2013). Museums hold vast collections of skeletons that are an untapped resource for pathology studies. In some cases, the information gained from studying skeletal pathology can support the post-mortem diagnosis of natural and anthropogenic causes of death.

The prevalence of skeletal pathology in Indo-Pacific bottlenose dolphins *Tursiops aduncus* in SA was 76%, which is high compared with most other studies of species in the family Delphinidae. A similar prevalence was reported for the tucuxi *Sotalia fluviatilis* (Fragoso 2001), although Fettuccia et al. (2013) reported only 26% for this species. Another 2 studies have been reported from South America. For the Peale's dolphin *Lagenorhynchus australis* the prevalence was 41% (San Martín et al. 2016). A wide range of prevalence (5 to 69%, n = 354) was reported amongst 12 species from South America, including the common bottlenose dolphin *Tursiops truncatus* (Van Bressem et al. 2007). For Pacific white-sided dolphins *Lagenorhynchus obliquidens* from the eastern North Pacific this prevalence was 54% (n = 243) (Walker et al. 1986).

The types of skeletal pathologies observed in our study of *T. aduncus* were similar to those reported for small cetaceans from Europe (Kompanje 1995a,b, 1996, 1999), South America (Van Bressem et al. 2006, 2007), North America (Alexander et al. 1989, DeLynn et al. 2011), New Zealand (Berghan & Visser 2000) and Australia (Dixon 1984). However, cranial infection by the nematode *Crassicauda* sp. was not observed in the present study. This parasite infection has been recorded in *T. truncatus* from South America (Van Bressem et al. 2007) and the offshore form of this species in the northwestern Atlantic Ocean (Mead & Potter 1995). The presence of *Crassicauda magna* has been confirmed in the pygmy sperm whale *Kogia breviceps* from SA (Jabber et al. 2015), a species that is believed to inhabit the offshore environment (McAlpine 2017). Because *T. aduncus* inhabits the inshore environment of SA, it may not be in contact with this parasite.

Lytic lesions are generally formed by an inflammatory reaction of bone and adjacent soft tissues (Van Bressem et al. 2007), the aetiology of which is probably related to microorganism infections, injuries causing necrosis, chemicals and/or embedded foreign bodies (Kompanje 1995a, 1999, Turnbull & Cowan 1999). *Brucella ceti* infection is well known to cause bone lesions in many species of cetacean and is found worldwide (Hernández-Mora et al. 2013). Osteomyelitis associated with *B. ceti* infection was found in *T. truncatus* from the US (Goertz et al. 2011)

as well as discospondylitis from the Mediterranean Sea (Isidoro-Ayza et al. 2014). Although there is no known link between this pathogen and bone lesions in *T. aduncus*, 53% of individuals from the Solomon Islands were positive for its antibodies (Tachibana et al. 2006). *Brucella* spp. has yet to be identified by PCR in Australian pinnipeds (Lynch et al. 2011) and no literature is available for cetaceans. Reported infectious agents from SA that may also cause lytic lesions in *T. aduncus* are dolphin morbillivirus, *Streptococcus* spp., *Pasteurella* spp. and *Cornynebacterium ulcerans* (Kemper et al. 2005, 2016). *Vibrio* spp., *Photobacterium* spp. and *Clostridium* spp. are opportunistic bacteria that have the potential to cause secondary infection and lytic lesions. These have been identified SA dolphins (data available from SAMA). Ankylosis is a severe form of lytic and degenerative lesion and was recorded in a few SA *T. aduncus*. However, post-mortem findings did not establish the causes.

Degenerative lesions of bone are mainly formed by repetitive and chronic stimulation (Van Bressem et al. 2007). These have a positive correlation with age, as shown in the present study. Van Bressem et al. (2007) reported that degenerative diseases including hyperostosis and ankylosis spondylosis were especially prevalent in *T. truncatus* from South America. In our study, there were only 2 examples of ankylosis spondylosis, both in adults. In a study of South American *S. fluviatilis*, 3 cases of vertebral ankylosis in the cervical region were reported in adults (Fettuccia et al. 2013).

Malformations form during ontogenesis in mammals (Millen & Woollam 1963). San Martín et al. (2016) reported many cases in the cervical and lumbar regions of the vertebral column of *L. australis*. In the carcasses of *T. aduncus* from SA, malformations were observed in different parts of the body, including the cervical but not the lumbar region. During field studies of Hector's dolphin *Cephalorhychus hectori* and *T. truncatus*, apparent spinal abnormalities were observed in live animals (Berghan & Visser 2000, Ambert et al. 2017), and some of these cases may have been caused by malformation. Three dolphin cases from SA (both alive and dead), not included in the present study, showed severe deformity in the tail stock (SAMA unpubl. data) that also may have involved malformation.

To our knowledge, fusion of sternal ribs has not previously been reported in cetaceans, but was found in several *T. aduncus* studied here. Fusion of the first 2 vertebral ribs in cetaceans has been reported in conjunction with pathology (Groch et al. 2012) and with anomalies related to function and evolution (Buchholtz 2011). DeLynn et al. (2011) reported fused 5th and 6th vertebral ribs in a *T. truncatus* from Florida. One example of vertebral rib malformation was observed in *T. aduncus* from SA, in the mid-thoracic region (T4, T5). Similar cases have been reported by Buchholtz (2011) and Groch et al. (2012).

Skeleton fractures are often related to interactions between fisheries and cetaceans (Duignan et al. 2003, Van Bressem et al. 2007). In SG, many of the dolphins that died as a result of anthropogenic circumstances had skeletal fractures (18/22), most of which were entanglements. SG supports many fishery and finfish aquaculture activities (Heaven et al. 2014) and entanglement is a known cause of death for *T. aduncus* there (Kemper & Gibbs 2001, Kemper et al. 2005, Gibbs & Kemper 2014). GSV has fewer fishing operations (Clarke & Madigan 2008) and did not show these patterns of pathology related to anthropogenic cause of death.

A female dolphin (M22410) from upper SG was a noteworthy case of skeletal pathology with unknown aetiology. This dolphin had clearly experienced trauma, as evidenced by the healed fractures on 3 neural spines in the lumbar region. However, the detached transverse processes did not appear to be related to this trauma because there was no callus formation typical of a fracture. In addition, the proximal ends of the transverse process were smaller than the neighbouring processes, which implies the pathology occurred when the dolphin was younger. Similarly, lytic and degenerative lesions can be ruled out because the surface of the bone was smooth. Further investigation of this case is required from the perspective of ontogeny and possible influence of heavy metal toxicity (see below).

Studies of cetacean skeletons have reported pathologies in all major regions of the body, with vertebral pathology commonly reported for small cetaceans, including *Tursiops* spp. (Alexander et al. 1989, DeLynn et al. 2011, Costa et al. 2016). The unique, simplified body structure of modern cetaceans results in the main locomotory force being the longissimus muscles and associated tendons that attach to the caudal vertebrae (Pabst et al. 1999). During locomotion, considerable mechanical stress is placed on vertebrae (Buchholtz & Schur 2004), particularly in the caudal region (Kompanje 1996). This stress may result in more pathology along the spine relative to other parts of the skeleton. Such a trend was observed in the *T. aduncus* studied from SA.

In our study, mature dolphins showed a higher prevalence of skeletal pathology than immature indi-

viduals, and all adults had some form of skeletal pathology. This relationship was also apparent for 3 types of pathology: lytic lesions, degenerative lesions and fractures. A similar pattern of age-related prevalence has been observed in other cetaceans. In white-beaked dolphins *Lagenorhynchus albirostris,* almost half of physically mature dolphins had fused vertebrae as a result of degenerative lesions (Galatius et al. 2009). Kompanje & Hartmann (2001) and Kompanje (2017) suggested that degeneration of the intervertebral discs and paravertebral ligaments, and herniation of intraspongious discs were the main contributors to age-related vertebral column pathology. Dental lesions showed more chronic and progressive stages with increasing age for several species of Delphinidae (Loch et al. 2011). The increased prevalence of skeletal pathology with age is contrary to what might be expected because bone mineral density increases with age (Butti et al. 2007). However, this may be overridden by the increased exposure to many factors (including pathogens and trauma) with time.

Coastal habitats are highly susceptible to bioaccumulation resulting from natural and anthropogenic inputs into the marine environment. This is particularly true for inverse estuaries such as the SA gulfs (Bye & Kämpf 2008). The northern part of SG and the area near Adelaide in GSV are known to be high in pollutants (Edwards et al. 2001). Lavery et al. (2008) found very high concentrations of some heavy metals in *T. aduncus* from the SA gulfs as well as preliminary evidence of bone and kidney pathology related to zinc, cadmium and lead (Lavery et al. 2009). These metals have been linked to bone metabolism and disruption of bone structure (Bremner 1974), and osteomalacia and osteoporosis have been linked to high heavy metal concentrations (Järup 2003). In the present study, some of the degenerative lesions may have included these pathologies as well as fractures. In addition, heavy metals can lead to malformation during an animal's development (Bremner 1974, Osada et al. 2011). One of the *T. aduncus* studied by Lavery et al. (2009) was also examined in the present study (M22410). This individual had high liver concentrations of cadmium (98 mg kg^{-1} wet weight) and zinc (209 mg kg^{-1} wet weight) and several other indicators of metal toxicity. Our study observed noteworthy abnormalities, possibly related to malformation, in 4 lumbar vertebrae of M22410. The aetiology of skeleton malformations has been linked to genetic diseases and acquired abnormalities, including nutritional imbalance and toxins (Thompson 2007).

Future research on SA bottlenose dolphins could compare skeletons from the gulfs with those collected along the open ocean coast, including *T. truncatus*, an offshore species. The 2 environments are likely to yield dissimilar results. Also, it would be interesting to compare *Tursiops* spp. with the common dolphin *D. delphis* in SA; SAMA has a large collection of the latter species. Comparison with soft tissues would enhance future studies of skeleton pathology of SA cetaceans.

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